Purification of restriction endonuclease *Eco*RII and its co-crystallization with DNA-substrate.

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Restriction endonuclease *Eco*RII (*Eco*RII) is a homodimeric DNA-binding protein. It belongs to the type II family of restriction-modification enzymes (subclass IIe). *Eco*RII recognizes the nucleotide sequence 5'-CCWGG (W=A or T) and cleaves the phosphodiester bond preceding the first cytosine. Methylation at C5 of the second cytosine inhibits cleavage. The enzyme has a unique ability to search for the presence of two substrate sites before cleavage.

To the best of our knowledge no other subclass IIe restriction endonuclease has been crystallized yet, without or with a DNA-substrate. We have recently grown and characterized the crystals of this enzyme (1) Here we report on the result of co-crystallization experiments of *Eco*RII with an 11 b.p. oligonucleotide substrate. The dissociation constant (Kd) *Eco*RII:11 b.p. was determined earlier (unpublished results). The needle-like crystals of oligonucleotide–*Eco*RII protein complex were obtained with this substrate by the technique of vapor diffusion hanging drops. The crystals obtained were washed and dissolved in an aliquot of 10 mM Tris-HCl buffer, pH=7.5. Running a portion of this solution on the SDS-gel indicated the presence of endonuclease in the solution. A UV-spectrophotometric test of a second portion confirmed the presence of DNA. We are now working on improvement of the DNA-*Eco*RII protein crystals. Results obtained from these and ongoing efforts will be reported.

REFERENCES:

1. Karpova, E., Meehan, E, Pusey, M. and Liqing Chen. Crystallization and preliminary X-ray diffraction analysis of restriction endonuclease *Eco*RII (1999) Acta Crystallographica, D55, 1604-1605.

Acknowledgments

The authors greatly thank the National Research Council of the National of Academy of Sciences for support in part of Dr. Elizaveta Karpova.